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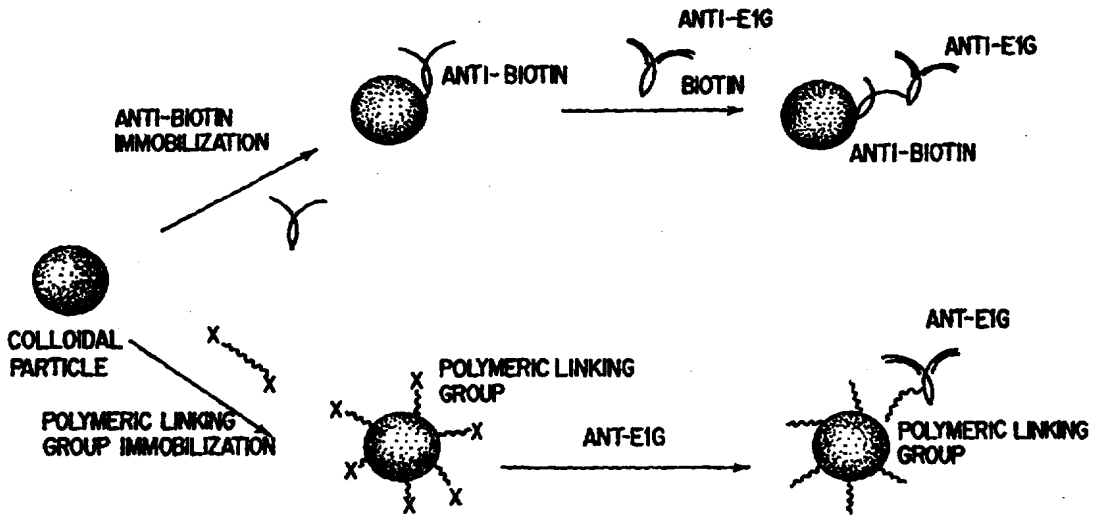

(57) Abstract

A label comprising a colloidal particle having a spacer arm attached thereto and a primary specific binding member attached to the spacer arm. The colloidal particle is preferably formed from a polymeric material or from a metal sol. The spacer arm is preferably a biological linking group or a synthetic polymeric linking group. The primary specific binding member is preferably an antibody or an antigen. The label of this invention exhibits a dose-response curve that varies only slightly over a wide temperature range, e.g., 13 °C to 37 °C when used in immunochromatographic assay strips.

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BINDING MEMBERS EXTENDING FROM PARTICLES FOR IMMUNOASSAY

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to colloidal particles that are useful for immunoassays, more particularly, immunochromatographic assays.

2. Discussion of the Art

Various analytical procedures can be used in diagnostic assays to determine the presence and/or amount of substances of interest or clinical significance in test samples, such as body fluids. These interesting or clinically significant substances are commonly referred to as analytes. Diagnostic assays have become an indispensable means for detecting analytes in test samples by using the reaction between the analyte and a specific binding member, as typified by the immunoreaction between an antigen and the antibody to that antigen.

In monitoring immunoreactions, use has been made of tags or labels composed of a traceable substance that is attached to a specific binding member, such as an antibody, which, in turn, binds to the analyte to form a labeled antibody/analyte complex. The detection of the labeled antibody/analyte complex, or of the labeled antibody that remains unbound, indicates the presence and/or amount of the analyte in the test sample.

Assay techniques using metallic sol particles as labels have been developed. In these techniques, particles of a metal (e.g., gold, silver, platinum), a metal compound, or a nonmetallic substance coated with a metal or a metal compound are used to form an aqueous dispersion of particles. The specific binding member to be labeled is coated onto the metallic sol particles by adsorption.

Particulate labels in immunoassay reagents have also been formed from polymeric dyes. Dye molecules, i.e., chromogenic monomers, are polymerized to form colored polymeric particles. The colored polymeric particles can then be linked to a specific binding member for use in an assay. Examples of such dyes include Congo red, Trypan blue, and Lissamine blue.

The dose-response curves of many assays are strongly affected by temperature when the colloidal particles used in the assay have specific binding members immobilized directly on the colloidal particles. This effect is a drawback for assays to be run under ambient conditions. For example, quantitative tracking of estrone glucuronide (hereinafter "E1G") in the urine of a female patient has been used to predict the fertility status of the patient. An immunochromatographic assay strip consisting of E1G immobilized on nitrocellulose and anti-E1G antibody immobilized on colloidal particles can be used to quantify E1G concentration in a competitive binding assay. Assay strips are described, for example, in U. S. Patent Nos. 5,120,643 and 5,252,459, incorporated herein by reference. However, the dose-response curve of E1G is strongly affected by temperature when the colloidal particles used in the assay have anti-E1G antibody immobilized directly on the colloidal particles. This effect is a drawback for the E1G assay when run under ambient conditions.

Many techniques have been tried to resolve the problem of temperature sensitivity. These techniques include the following:

(1) increasing or decreasing the antibody loading on the colloidal particle;

(2) internal correction design of patient bar with respect to the control bar;

(3) different derivatives of estrone for signal capture;

(4) devices on the diagnostic instrument for controlling temperature;

(5) thermistor to relay correction factors of the calibration curve.

The first three techniques have not resolved the problem resulting from temperature variation. The fourth technique is feasible but excessively costly. The fifth technique is workable only over a narrow temperature range. Therefore, it would be desirable to provide a simple technique for stabilizing the dose-response curve of E1G for an assay for E1G.

SUMMARY OF THE INVENTION

This invention provides a label comprising a colloidal particle having a spacer arm attached thereto and a specific binding member attached to the spacer arm. The label is particularly useful as an indicator reagent in immunoassays.

The colloidal particle is preferably formed from a polymeric material or from a metallic sol. A preferred material for the colloidal particle is polypyrrole.

The spacer arm is preferably a biological linking agent or a synthetic polymeric linking agent. The spacer arm preferably has a high molecular weight, e. g., greater than about 1,000.

The specific binding member is preferably an antibody or an antigen. An example of a preferred material for the specific binding member is anti-E1G antibody.

The label of this invention exhibits a dose-response curve that varies only slightly over a wide temperature range, e.g., 13 °C to 37 °C when used in immunochromatographic assay strips.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 schematically illustrates anti-E1G antibody attached to a colloidal particle via a spacer arm.

FIG. 2 illustrates one configuration of a strip suitable for use in the present invention.

FIG. 3 is a graph illustrating how assay results for a conventional antibody vary as a function of temperature.

FIG. 4 is a graph illustrating how assay results for an extended antibody of this invention vary as a function of temperature.

FIG. 5 is a graph illustrating how assay results for an extended antibody of this invention vary as a function of temperature.

FIG. 6 is a graph illustrating how assay results for an extended antibody of this invention vary as a function of temperature. The assay used for preparing this figure employed an assay strip.

FIG. 7 is a graph illustrating how assay results for an extended antibody of this invention vary as a function of temperature. The assay used for preparing this figure employed an assay strip.

FIG. 8 is a graph illustrating how assay results for an extended antibody of this invention vary as a function of temperature. The assay used for preparing this figure employed an assay strip.

DETAILED DESCRIPTION OF THE INVENTION

The following terms and expressions will be used to describe the invention.

"Analyte," as used herein, means the substance to be detected in a test sample. An analyte can be any substances for which there exists a naturally occurring specific binding member (e.g., an antibody) or for which a specific binding member can be prepared. The analyte can bind to one or more specific binding members in an assay. "Analyte" also includes any antigenic substances, haptens, antibodies, and combinations thereof. The analyte can include a protein, a peptide, an amino acid, a hormone, a steroid, a vitamin, a drug including those administered for therapeutic purposes as well as those administered for illicit purposes, a bacterium, a virus, metabolites of or antibodies to any of the above substances, nucleic acids, and ribonucleic acids.

"Specific binding member," as used herein, means a member of a specific binding pair. A specific binding pair comprises two different molecules, wherein one of the molecules through chemical or physical means specifically binds to the other molecule. An example of a specific binding pair is the antigen/antibody specific binding pair. In addition to antigen and antibody-specific bindings pairs, other specific binding pairs include, but are not limited to, biotin and avidin, carbohydrates and lectins, complementary nucleotide sequences (including probe and capture nucleic acid sequences used in DNA hybridization assays to detect a target nucleic acid sequence), complementary peptide sequences, effector and receptor molecules, enzyme cofactors and enzymes, enzyme inhibitors and

enzymes, and the like. Furthermore, specific binding pairs can include members that are analogues of specific binding members originally contemplated. For example, a derivative or fragment of the analyte (i.e., an analyte-analogue) can be used so long as it has at least one epitope in common with the analyte. Immunoreactive specific binding members include antigens, haptens, antibodies, and complexes thereof, including those formed by recombinant DNA methods or peptide synthesis.

"Indicator reagent," as used herein, means a detectable material, in particulate form, directly or indirectly attached to a specific binding member.

"Capture reagent," as used herein, means a specific binding member capable of binding the analyte or indicator reagent. The capture reagent can be directly or indirectly attached to a substantially solid material to form a solid phase capture reagent complex. The solid phase capture reagent complex can be used to separate the bound and unbound components of the assay.

The label of this invention comprises a colloidal particle having a spacer arm attached thereto and a specific binding member attached to the spacer arm. In effect, the specific binding member is extended from the surface of the colloidal particle by means of the spacer arm.

Colloidal particles suitable for use in preparing the label of this invention can be made of polymeric material, metallic material, non-metallic material, aggregated dye material, and other materials conventionally used to prepare colloidal particles for use in immunoassays. Materials that are preferred for the colloidal particles include polymers having pyrrole units, e.g., polypyrrole, polymers having styrene units, polymers having acrylate units, and colloidal metals, e.g., colloidal gold, colloidal selenium. The size of the colloidal particles can range from about 10 nm to about 5,000 nm in average diameter.

Polymeric particles suitable for use in the present invention can be made by polymerizing a nonchromophoric monomer; nonchromophoric monomer, as used herein, refers to an organic monomer that is neither a pigment nor a dye and that has color or absorbance characteristics that make the unpolymerized substance unsuitable for use as a detectable label. The polymeric particles absorb light in the visible, ultraviolet, or infrared regions at one or more wavelengths at which the nonchromophoric monomer does not. The absorbance characteristics of the polymeric particle result from the extensively conjugated structure arising from the polymerization process. Thus, the polymeric particle derives its characteristic color or absorbance from its polymeric structure rather than from the color or absorbance

of the monomer from which it is made or from the addition of a dye or a pigment, i.e., a chromogen. For example, pure monomeric pyrrole is a substantially nonchromophoric substance, but the poly(pyrrole) latex particle is black, due to its extensively conjugated structure and unpaired delocalized electrons (stable radicals). As used herein, "substantially nonchromophoric" means that impurities that impart a color to the monomer may be present, although typically, the pure form of the monomer is transparent or colorless.

The polymeric latex particles of this invention can be divided into at least six classes. The first class comprises polymeric particles made from a single nonchromophoric monomeric material. Such polymeric particles include, but are not limited to, particles made from the following polymers: poly(pyrrole), polyacetylene, polyphenylene, poly(aniline), poly(thiophene), poly(naphthalene), poly(thiophenol), their derivatives, such as poly(n-methyl pyrrole), poly(n-benzyl pyrrole), poly(n-phenyl pyrrole) [e.g., poly(1-phenyl 2,5 pyrrolene)] and poly(p-phenylene diethynyl), i.e., -(-C≡C-C_6H_4C≡C-)-, and their equivalents.

The second class of polymeric particles comprises a composite of both nonchromophoric monomers, which, upon polymerization, form a colored polymer, and nonchromophoric monomers which, upon polymerization, generally do not form a colored polymer. Composite particles can be formed by combining one of the above-described polymers, or oligomers thereof, with moldable polymeric materials, such as poly(vinyl chloride), polystyrene, and poly(vinyl toluene), as well as hydrophilic polymeric materials, such as poly(acrylamide) and poly(N-vinyl pyrrolidone), and derivatives thereof. The composite particles of blended materials may facilitate processing of the particle labels.

The third class of polymeric particles includes polymeric particles having repeating units of at least two different nonchromophoric monomers. Thus, copolymers can be formed and used as labels according to the present invention. Examples of copolymers include, but are not limited to, poly(pyrrole-co-thiophene) and poly(pyrrole-co-phenylene). The organic monomers include: pyrrole, benzene, toluene, aniline, thiophene, naphthalene, thiophenol and equivalent aromatic monomers, acetylene and equivalent nonaromatic monomers, and derivatives thereof.

The fourth class of polymeric particles comprises an organic or inorganic nucleus that can be coated with a polymer made by the polymerization of a plurality of nonchromophoric monomers. For example, a metallic colloidal particle can be coated with the colored organic polymeric latex.

The fifth class of polymeric particles comprises a polymeric particle made of a plurality of nonchromophoric monomers polymerized to form a substantially colorless polymeric particle which, upon further reaction with a substance that is not a dye or pigment, will become a colored polymeric particle. An example of such a polymeric particle is poly(phenylene sulfide), a normally transparent and substantially colorless polymeric material, which becomes blue-green and finally blue-black upon further reaction with arsenic pentafluoride.

The sixth class of polymeric particle comprises a polymeric particle made of a plurality of nonchromophoric monomers which, upon polymerization, form a particle having detectable free radicals, and the resulting particle can be colored or substantially colorless. While such colorless particles may not be visually detectable, the stable free radical nature of these particles enables their detection and measurement with an electron paramagnetic resonance spectrometer.

Spacer arms suitable for use in preparing the label of this invention can be made of biological linking groups or synthetic polymeric linking groups. Materials that are preferred for the spacer arm include antibodies, protein A, protein G, avidin, biotin, hydrophilic polymers, such as, for example, polymers having vinyl alcohol units, e.g., polyvinyl alcohol, polymers having acrylate units, e.g., hydroxy alkyl acrylate polymers, polymers having alkylene glycol units, e.g., polyethylene glycol, polymers having amide units, e.g., polyamides, polymers having peptide units, e. g., polypeptides, polymers having saccharide units, e.g., polysaccharides, polymers having nucleotide units, and the like. The spacer arm must be sufficiently long to minimize steric hindrance resulting from the surface of the colloidal particle; however, the spacer arm must not be so long that non-specific entangling will result. Preferably, the length of the spacer arm ranges from about 5 nm to about 100 nm. The spacer arm preferably has a high molecular weight, e.g., greater than about 1,000. The spacer arm can be a single entity or a combination of two or more entities linked together, preferably by covalent bonds. However, it is much simpler to use a single entity as a spacer arm.

Specific binding members suitable for use in preparing the label of this invention can be made of specific binding proteins. Materials that are preferred for the specific binding member include antibodies, enzymes, receptors, antigens, haptens, and epitopes.

It is relatively easy to attach specific binding members to spacer arms attached to colloidal particles by covalent bonds to form indicator reagents useful

in the present invention. The specific binding member can be attached to the spacer arms attached to the colloidal particles either by adsorption, a linking group, or a surface-functional group. Covalent attachment of colloidal particles to spacer arms can be accomplished by using carboxylic, amino, aldehyde, bromoacetyl, iodoacetyl, thiol, epoxy, and other reactive or linking groups, as well as by using residual free radicals and radical cations, through which a protein coupling reaction can be accomplished.

The label of this invention can be prepared by applying a layer of material suitable for forming spacer arms to the surface of the colloidal particle. The material for preparing the spacer arms can be adsorbed onto the surface of the colloidal particle. Adsorption can be enhanced by chemical treatment. For example, the addition of periodate to the colloidal particle enhances adsorption of spacer arms to the colloidal particle. Preferably, spacer arms become attached to the colloidal particle by means of crosslinking of aldehyde groups generated from the oxidation resulting from addition of periodate to the colloidal particle.

The colloidal particle to which is attached the spacer arms can be treated with the specific binding member. The specific binding member can be attached to a spacer arm by immunocomplex formation or covalent linkage. Preferably, the specific binding member becomes attached to the spacer arm by means of immunocomplex formation.

FIG. 1 illustrates in schematic form how the label of the present invention can be prepared. In one embodiment, a component of a spacer arm, e.g., anti-biotin, is immobilized on the surface of a colloidal particle. Then, a specific binding member, e.g., an antibody, such as, for example, anti-E1G antibody, attached to an entity capable of specifically binding to the component of the spacer arm, e.g., biotin, is attached to the component of the spacer arm to extend the specific binding member from the surface of the colloidal particle. In a second embodiment, a spacer arm, e.g., a polymeric material, is immobilized on the surface of a colloidal particle. Then, a specific binding member, e.g., an antibody, such as, for example, anti-E1G antibody, is attached to the spacer arm to extend the specific binding member from the surface of the colloidal particle.

The size of the colloidal particles can be varied in order to provide better assay results. The colloidal particles preferably range in size from about 10 to about 5,000 nm, more preferably from about 50 nm to about 1,000 nm.

Size of the colloidal particles can be controlled by using appropriate conditions for polymerization in the formation of the raw colloidal particles or by

using an excess of specific binding member to prevent aggregation of the colloidal particles or by employing both conditions. The colloidal particles can be washed by diafiltration, rather than by centrifugation. Further reduction of size of the colloidal particles can be achieved by sonication.

The label of this invention is preferably stored in a medium to protect specific reactivity of the specific binding member and to prevent nonspecific binding. The medium may comprise proteins, carbohydrates, hydrophilic polymers, surfactants, salts, including buffer salts, and preservatives.

An assay device suitable for use with the present invention can have many configurations, several of which are dependent upon the material chosen for the solid phase. Preferably, the solid phase material can be any suitable chromatographic, bibulous, or porous material. Solid-phase materials suitable for this invention include, but are not limited to, (1) a fiberglass, cellulose, or nylon pad for use in a flow-through assay device having one or more layers, containing one or more of the assay reagents, (2) a dipstick for a dip and read assay, (3) a test strip for chromatographic techniques (e.g., paper or glass fiber) or thin layer chromatographic techniques (e.g., nitrocellulose), in which the reagents are contained in separate zones of a single strip of solid phase material, or (4) an absorbent material well known to those skilled in the art. The solid phase material can also include, but is not limited to, polyacrylamide beads, polystyrene beads or tubes, magnetic beads, a microtiter plate, and a glass or plastic test tube.

Natural, synthetic, or naturally-occurring materials that are synthetically modified can be used as a solid phase material. The materials include polysaccharides, e.g., cellulose materials such as paper and cellulose derivatives such as cellulose acetate and nitrocellulose; silica; silicon particles; inorganic materials, such as deactivated alumina; or other inorganic particulate material uniformly dispersed in a porous polymeric matrix, with polymers such as, for example, vinyl chloride, copolymers of vinyl chloride and propylene, and a copolymer of vinyl chloride and vinyl acetate; cloth, both naturally occurring (e.g., cotton) and synthetic (e.g. nylon); porous gels such as silica gel, agarose, dextran, and gelatin; polymeric films such as polyacrylates; protein binding membranes; and the like. The solid phase material should have sufficient strength for immunoassay purposes or sufficient strength can be provided by means of a support. The solid phase material should not interfere with the production of a detectable signal.

The labels of the present invention can be used in a variety of assay formats. In general, any assay configuration using specific binding members and a detectable label, such as, for example, organic polymeric latex particles, can be used.

Specific binding assays are generally categorized into one of two major classes, homogeneous and heterogeneous assays. In a homogenous assay, the reaction components or reagents are in the test solution and are not separated prior to the detection of the signal produced by the indicator reagent. In a heterogeneous assay, either a solid phase material is used to allow the separation of bound from unbound reaction components, or a reagent of the initial solution is caused to precipitate and is subsequently removed from the test solution. The indicator reagent of the present invention is detectable in both its bound and unbound forms and can be used in both homogeneous and heterogeneous assays. These assays may be further divided into sandwich and competitive assays, and variations thereof.

The label of this invention can be used as an indicator reagent in a competitive assay format in an immunochromatographic assay strip. A typical assay strip can comprise a bibulous nitrocellulose membrane through which colloidal particles of sub-micron size can flow by capillary action. The colloidal label will be captured by capture reagent immobilized on the nitrocellulose membrane. The binding reaction between the analyte and the immobilized capture reagent will compete with the binding reaction between the colloidal label and the immobilized capture reagent on the membrane. When the label accumulates at a detection zone, the zone will appear to be colored. The optical density of the detection zone can be measured with a transmittance or reflectance light scanner that compares the optical density of the detection zone with background and a white reference standard. In some situations, results can be determined visually. Computer driven micro-jetting process can be used to achieve penetration and homogeneous distribution of the capture reagent. A typical assay strip will be described in greater detail in the working examples.

The label can be used for a self-performing assay with assay strips designed for immunoassays. The label can be used in two modes --- the wet mode and the dry mode. In the wet mode the label is mixed with the sample, the mixture is transferred to one end of the membrane strip directly or through a porous pad, and the label will flow spontaneously through the test strip. Typically, the test will be completed within 10 minutes, and often within five minutes. The results can be

read either visually for qualitative results or instrumentally for quantitative results. However, it is preferable to use the label in the dry mode. The label can be impregnated in a porous pad, dehydrated via lyophilization or dry air, attached to one end of a membrane strip, and covered with an overseal or case into an assembled test strip. The assembled assay strip is a miniature test kit including all essential reagent components. The user needs only to add the test sample to the pad and wait a few minutes for the test result.

The present invention allows the manufacturer of self-performing assays and automated assays performable via instruments to provide a diagnostic assay that is capable of generating consistent results, regardless of variations in environmental conditions, such as temperature and humidity. The label of this invention exhibits a dose-response curve that varies only slightly over a wide temperature range, e.g., 13 °C to 37 °C when used in immunochromatographic assay strips. Prior to this invention, dose-response curves varied as much as fifty-fold or greater over a wide temperature range, e.g., 13 °C to 37 °C when used in immunochromatographic assay strips. This invention reduces variation in dose-response curves over the aforementioned temperature range to no more than five-fold, and often no more than three-fold.

The following non-limiting examples will further illustrate the present invention. The following commercially available materials were used in the examples:

MES:	2-[N-morpholino]ethanesulfonic acid
MOPS:	3-[N-morpholino]propanesulfonic acid
BIS-TRIS:	bis[2-hydroxyethyl]iminotris[hydroxymethyl]methane
Brij 35 solution:	polyoxyethylene ether
BSA:	bovine serum albumin

EXAMPLES

Example I

Preparation Of Biotinylated Anti-E1G Antibody

A solution containing monoclonal anti-E1G antibody (1.5 mg antibody in 750 µL of 0.1 M sodium bicarbonate, pH 8.3) was magnetically stirred. A solution containing biotin-amidocaproylamidocaproate N-hydroxysuccinimide ester (0.065 mg ester in 20 µL of dimethylformamide, freshly dissolved) was added to the stirred antibody-containing solution in a dropwise manner. The resulting solution was mixed at ambient temperature for two hours, transferred to a dialysis tube (Sigma Chemical Company, molecular weight cutoff 12,000), and then dialyzed in phosphate buffered saline (10 mM sodium phosphate, 0.85% sodium chloride, hereinafter "PBS") in a one liter flask with three volume exchanges of buffer. The dialyzed antibody was filtered (0.2 micron filter), and optical density (wavelength at 280 nm) was checked to calculate concentration of IgG antibody. The final volume was 10 mL with a concentration of antibody of 0.58 mg/mL. The incorporated biotin was quantified by a fluorescence polarization immunoassay (FPIA) method on a TDx® instrument (Abbott Laboratories), and the biotin-to-IgG molar ratio was 2.0. A preservative (Proclin-300®, Rohm and Haas) was added at a concentration of 0.1% to the final solution of biotinylated anti-E1G antibody.

Example II (Comparative)

Preparation Of Anti-E1G Antibody Immobilized On Polypyrrole Colloid

Monoclonal anti-E1G antibody (2 mg) was mixed with polypyrrole colloid (0.075% solids) in borate buffer (5 mL, 20 mM borate, pH 10, 0.2% Brij 35 solution) at a temperature of 45 °C (water-bath) for two hours. To the reaction mixture was added BIS-TRIS/BSA buffer (1.25 mL, 0.25 M BIS-TRIS, pH 7.0, 4% BSA, 0.1% Brij 35 solution), and the resulting solution stirred for 10 minutes. The reaction mixture was mixed for three hours at a temperature of 2 to 8 °C, then 0.3 M H_5IO_6 (555 µL, Aldrich Chemical Company), freshly dissolved in 0.5 M triethanolamine was added to the reaction mixture, and the resulting mixture

mixed overnight. The colloidal particles were washed with a MOPS/BSA buffer (25 mM MOPS, pH 7.2, 25 mM ethanolamine, 2% BSA, 0.1% Brij 35 solution, 0.05% Proclin-300® preservative) on a miniature diafiltration washing device (Microgon®), and finally dispersed in a MES/casein diluent (0.1 M MES, pH 6.1, 5% hydrolyzed casein, 0.024% Triton® surfactant, 0.04% bovine IgG, 0.1% Proclin-300® preservative). The concentration of colloid was adjusted by light scattering to an optical density of 4 (wavelength at 560 nm).

Example III

Preparation Of Anti-biotin Polypyrrole Colloid

Monoclonal anti-biotin IgG antibody (0.225 mg) was mixed with polypyrrole colloid (0.75% solids) in borate buffer (1 mL, 20 mM borate, pH 10, 0.2% Brij 35 solution) at a temperature of 45 °C (water-bath) for two hours. To the reaction mixture was added BIS-TRIS/BSA buffer (250 µL, 0.25 M BIS-TRIS, pH 7.0, 4% BSA, 0.1% Brij 35 solution) and the resulting mixture stirred for 10 minutes. The reaction mixture was mixed for three hours at a temperature of 2 to 8 °C, then 0.3 M H_5IO_6 (111 µL, Aldrich Chemical Company), freshly dissolved in 0.5 M triethanolamine was added to the reaction mixture, and the resulting mixture mixed overnight. The colloidal particles were spun down, washed with a MOPS/BSA buffer (25 mM MOPS, pH 7.2, 25 mM ethanolamine, 2% BSA, 0.1% Brij 35 solution, 0.05% Proclin-300® preservative), exchanged with wash buffer through two centrifugations and sonications, and finally dispersed in MOPS/BSA buffer.

Example IV

Preformed Immune Complex Of Anti-E1G Antibody/Anti-Biotin Polypyrrole Colloid

Biotinylated anti-E1G IgG antibody from Example I (800 µL, 0.464 mg) was introduced into 200 µL of the solution of anti-biotin colloid from Example III with 1 mL of the MOPS/BSA buffer, and the resulting mixture stirred at ambient temperature for 2 1/2 hours. The colloid particles were spun down, washed with

MOPS/BSA buffer, exchanged with wash buffer through two centrifugations and sonications, and finally dispersed in a MES/casein diluent (0.1 M MES, pH 6.1, 5% hydrolyzed casein, 0.024% Triton® surfactant, 0.04% bovine IgG, 0.1% Proclin-300® preservative). The concentration of colloid was adjusted by light scattering to an optical density of 4 (wavelength at 560 nm).

Example V

Preparation Of Goat Anti-Mouse IgG Antibody Polypyrrole Colloid

Goat anti-mouse IgG antibody (0.53 mg) was mixed with polypyrrole (1% solids) in borate buffer (4 mL, 20 mM borate, pH 10, 0.2% Brij 35 solution) at a temperature of 45 °C (water-bath) for two hours. To the reaction mixture was added BIS-TRIS/BSA buffer (1 mL, 0.25 M BIS-TRIS, pH 7.0, 4% BSA, 0.1% Brij 35 solution), and the mixture stirred for 10 minutes. The reaction mixture was mixed for three hours at a temperature of 2 to 8 °C, then 0.3 M H_5IO_6 (555 µL, Aldrich Chemical Company), freshly dissolved in 0.5 M triethanolamine was added to the reaction mixture, and the resulting mixture mixed for 18 hours. The colloidal particles were washed on a diafiltration washing device (Microgon®), washed with a MOPS/BSA buffer (25 mM MOPS, pH 7.2, 25 mM ethanolamine, 2% BSA, 0.1% Brij 35 solution, 0.05% Proclin-300® preservative), with 10 volume exchanges with buffer.

Example VI

Preformed Immune Complex Of Anti-E1G Antibody/Goat Anti-Mouse Polypyrrole Colloid

Colloid coated with goat anti-mouse antibody from Example V (1 mL) was added to monoclonal anti-E1G antibody in MOPS/BSA buffer (400 µg antibody/mL of buffer) as above. The colloid was incubated overnight, and, without further wash, was diluted with the MES/casein diluent as Example IV to give a concentration corresponding to an optical density of 4 (wavelength of 560 nm).

Example VII

Preformed Immune Complex Of Anti-E1G Antibody/ Anti-Biotin Polypyrrole Colloid Air Dried On Lydall® Pad.

Various embodiments of the assay strip suitable for use with this invention include different configurations of functional zones. Some of these embodiments are shown in U. S. Patent Nos. 5,120,643 and 5,252,459, incorporated herein by reference.

In the embodiment used in this example and illustrated in FIG. 2, an assay strip 10 includes a porous strip material 12 on which are located a sample mixing zone 14, a first capture zone 16, and a second capture zone 18. The sample mixing zone 14 contains dried indicator reagent particles. The indicator reagent particles contain first binding members that specifically bind to the analyte of interest. The first capture zone 16, termed the "patient bar," is a region of the porous strip material 12 in which the extent of reaction is a function of the concentration of the analyte of interest. More specifically, the first capture zone 16 contains second binding members attached to the strip material 12 that specifically bind to the first binding members. The second binding members compete with the analyte of interest for binding sites on the first binding member. The extent of binding may be indicated by the level of optical reflectance at the first capture zone 16. A second capture zone 18, termed the "control bar," is also present. In the control bar, the extent of binding is relatively insensitive to the concentration of the analyte of interest. More specifically, the second capture zone 18 contains third binding members attached to the porous strip material 12 that bind to the first binding members on the indicator reagent particles at sites unrelated to the analyte of interest. The extent of binding in the patient bar 16 and the control bar 18 may be sensitive to factors such as the temperature at which the assay is performed and the age of the reagents within the porous strip material 12. The function of the control bar is to indicate whether the reagents have deteriorated during storage. The extent of binding in the control bar is inversely proportional to the level of deterioration. The method of indicating the extent of binding in the second capture zone 18 will normally be the same as that for the first capture zone 16, although this is not necessary.

The assay utilizing the assay strip 10 shown in FIG. 2 is initiated when a known quantity of a liquid sample of a body fluid, such as a urine sample, suspected of containing the analyte of interest, is deposited on the sample mixing zone 14 of the porous strip material 12. The first binding members on the indicator reagent particles tend to bind to the analyte of interest to an extent that is approximately proportional to the concentration of that analyte in the liquid sample. The liquid sample flows through the porous strip material 12, carrying with it both the reacted (bound) and unreacted (unbound) first binding members on the indicator reagent particles. In this embodiment, the expression "flows through" means flows laterally through the strip material via the capillaries in the porous strip material. In another type of embodiment, the expression "flow-through" refers to an assay device wherein a flowable material flows through one or more layers of material. As used herein, the expressions "flow through", "flows through", and the like is intended to encompass all embodiments, whether they involve lateral flow or flow from one layer through another layer. At the first capture zone 16, i.e., the patient bar, indicator reagent particles that carry sufficient pluralities of unreacted first binding members tend to bind the second binding members fixed to the porous strip material 12. Therefore, the extent of binding between the indicator reagent particles and the assay strip at the first capture zone is a function of the concentration of the analyte of interest in the sample of body fluid; the higher the concentration of the analyte of interest, the lower the extent of binding of the indicator reagent particles at the first capture zone. The body fluid sample continues to flow through the porous strip material 12, carrying with it the remainder of the indicator reagent particles, to the second capture zone 18, i.e., the control bar. At the control bar 18 are the third binding members fixed to the porous strip material 12 and that tend to bind to the first binding members on the indicator reagent particles. The amount of binding at the control bar 18 is indicative of the condition of the assay and reagents, and may be used to adjust the quantification of binding at the patient bar 16 to correct for assay temperature and reagent aging.

Preformed immune complex of anti-E1G antibody/anti-biotin polypyrrole colloid as described in Example IV was diluted in a MES/casein/sugar diluent (0.1 M MES, pH 6.1, 2.5% hydrolyzed milk, 2% Trehalose, 0.012% Triton® surfactant, 0.02% bovine IgG, 0.05% Proclin-300® preservative). The concentration of colloid was adjusted to correspond to an optical density of 2 (wavelength of 560 nm). The colloid was introduced into a long strip (13 mm in

width) of fiberglass pad (Lydall®), 2 mL of solution per foot. The colloid impregnated strip was dried by being passed through a tunnel with flow of warm air. Then the assay strip was assembled with ribbons of nitrocellulose membrane, onto which were jetted a patient bar containing E1G-bovine IgG conjugate and a control bar containing goat anti-mouse antibody. The assembled assay strip was cut into narrower strips having a width of 4 mm. Each assay strip 10, which is illustrated in FIG. 2, consisted of: (1) a strip 12 of nitrocellulose membrane, 4 mm x 30 mm; (2) a Lydall® pad 14 (4 mm x 13 mm) containing dried colloid, with 2.5 mm of the pad overlapping the end of nitrocellulose membrane; (3) a Patient Bar 16 containing E1G-bovine IgG conjugate (0.1 µL at a concentration of 5 mg/mL, 50 mM borate, pH 8.5) disposed on the nitrocellulose membrane 10 mm from the Lydall® pad; (4) a Control Bar 18 containing goat anti-mouse antibody (0.1 µl at a concentration of 0.83 mg/mL, PBS, pH 7.2, 0.2% sucrose) disposed on the nitrocellulose membrane 20 mm from the Lydall® pad, (5) a transparent laminate 20 covering the nitrocellulose membrane and partially covering the Lydall® pad, and (6) a small sheet of plastic 22 for holding the assembled nitrocellulose strip and Lydall® pad in place. The purpose of the transparent laminate is to prevent moisture from adversely affecting the reagents on the nitrocellulose membrane and on the Lydall® pad.

Example VIII

Competitive Binding Assay Of E1G in Urine On Test Strip At Three Temperature Levels In Wet Mode

An E1G assay strip was assembled in a manner similar to that used in Example VII except that a blank Lydall® pad was used in place of the Lydall® pad impregnated with dried colloid. The strip assay was run on a strip reader, which was a reflectance scanner. The strip reader was able to handle six strips in parallel and was controlled through a personal computer with data collection capability. The protocol for running the assay was as follows: (1) 25 µL of a E1G urine calibrator was transferred to a disposable 1 mL vial, (2) 25 µL of colloid containing anti-E1G antibody was transferred to a vial, (3) the contents of the vials were mixed, and immediately, 40 µL of the mixture were transferred to the Lydall® pad of a strip that was set on the reader. The running time of each

individual assay was about five minutes, and the complete cycle of testing and data processing on the reader took about 10 minutes for each run.

Assay results using colloids containing anti-E1G antibody from Example II, Example IV, and Example VI with E1G calibrators are shown in FIG. 3, FIG. 4, and FIG. 5, respectively. FIG. 3 indicates that the dose-response of anti-E1G antibody immobilized on polypyrrole colloid varied significantly with respect to temperature. FIG. 4 indicates that the dose-response of anti-E1G antibody immobilized on polypyrrole colloid via the anti-biotin spacer arm varied little with respect to temperature. FIG. 5 indicates that the dose-response of anti-E1G antibody immobilized on polypyrrole colloid via the goat anti-mouse antibody spacer arm varied little with respect to temperature.

Example IX

Competitive Binding Assay Of E1G in Urine On Test Strip At Three Temperature Levels In Dry Mode

The E1G assay strip used in this example was assembled in the manner as was used in Example VII. The strip assay was run on the same strip reader as described in Example VII. There was no need for sample mixing. A sample (40 µL) was simply added to the exposed end of Lydall pad of each strip set on the reader. FIG. 6 shows test results of E1G calibration curves generated at three different temperatures --- 13 °C, 25 °C, and 37 °C. FIG. 6 indicates that the dose-response of anti-E1G antibody immobilized on polypyrrole colloid via the anti-biotin spacer arm varied little with respect to temperature when used in the dry mode type of assay.

Example X

Anti-E1G Antibody Linked By Bis[polyoxyethylene bis(amine)] And Glutaraldehyde Spacer Arm To Polypyrrole Colloid

Bis(polyoxyethylene bis[amine]) (1 mg, molecular weight 20,000, Sigma Chemical Company) was mixed with polypyrrole colloid (0.76% solids) in borate

buffer (5 mL, 20 mM borate, pH 10, 0.2% Brij 35 solution) at ambient temperature for three hours. The reaction mixture was mixed for 10 minutes at a temperature of 2 to 8 °C. Then 0.3 M H_5IO_6 (555 µL), freshly dissolved in 0.5 M triethanolamine was added to the reaction mixture, and the resulting mixture mixed overnight. The colloid was bath-sonicated for 30 minutes to break up visible clumps. The colloid was washed with phosphate buffer (20 mM sodium phosphate, pH 8.4, 0.1% Brij 35 solution) on a miniature diafiltration washing device (Microgon®), and colloid containing bis(polyoxyethylene bis[amine]) (10 mL) was recovered. The recovered colloid (2.5 mL) was slowly added to a vigorously mixed solution of diluted glutaraldehyde (0.3% in H_2O, 2.5 mL), and the resulting mixture stirred at a temperature of 2 to 8 °C overnight. The resulting colloid was washed and exchanged with phosphate buffer (20 mM sodium phosphate, pH 7.1, 0.1% Brij 35 solution) on the miniature diafiltration washing device (Microgon®); 3.5 mL of colloid was recovered; the recovered colloid was added to 1 mL of anti-E1G antibody (3 mg of antibody in 0.1 M phosphate, pH 10.0, 0.1% Brij 35 solution). The pH at the conclusion of mixing was 7.5; then, the colloid was added to sodium cyanoborohydride (4.5 mg in H_2O, 100 µL), and the resulting mixture was mixed at a temperature of 2 to 8 °C overnight. The resulting colloid was then mixed with 1 mL of BIS-TRIS/BSA buffer (0.25 M BIS-TRIS, pH 7.0, 4% BSA, 0.1 % Brij 35 solution) for 1 hour, and then washed and exchanged with the MOPS/BSA buffer (25 mM MOPS, pH 7.2, 25 mM ethanolamine, 2% BSA, 0.1% Brij 35 solution, 0.05% Proclin-300® preservative). The concentration of colloid coated with anti-E1G antibody was adjusted to an optical density of 25 (wavelength at 560 nm) in MOPS/BSA for storage.

Example XI

Anti-E1G Antibody Linked By Bis(polyoxyethylene bis[amine]) And Polyoxyethylene bis(p-nitrophenyl carbonate) Spacer Arm To Polypyrrole Colloid

Colloid containing bis(polyoxyethylene bis[amine]) (2.5 mL) prepared as described in Example X was slowly added to a vigorously mixed solution of polyoxyethylene bis(p-nitrophenyl carbonate) (7.5 mg in H_2O, 2.5 ml); the pH of the mixture was adjusted to 8.4, and the mixture stirred at a temperature of 2 to 8 °C overnight. The resulting colloid was washed and exchanged with phosphate

buffer (20 mM sodium phosphate, pH 7.1, 0.1% Brij 35 solution) on a miniature diafiltration washing device (Microgon®); 3.5 mL of colloid was recovered; the recovered colloid was introduced into 1 mL of anti-E1G antibody (3 mg of antibody in 0.1 M phosphate, pH 10.0, 0.1% Brij 35 solution), the pH was adjusted to 9.5, and the resulting mixture mixed gently at a temperature of 2 to 8 °C overnight. The resulting colloid was then mixed with BIS-TRIS/BSA buffer (1 mL, 0.25M BIS-TRIS, pH 7.0, 4% BSA, 0.1 % Brij 35 solution) for 1 hour and then washed and exchanged with MOPS/BSA buffer (25 mM MOPS, pH 7.2, 25 mM ethanolamine, 2% BSA, 0.1% Brij 35 solution, 0.05% Proclin-300® preservative). The concentration of colloid containing anti-E1G antibody was adjusted to an optical density of 46 (wavelength at 560 nm) in the MOPS/BSA buffer for storage.

Example XII

Competitive Binding Assay Of E1G In Urine On Test Strip At Three Temperature Levels In Wet Mode.

The E1G assay strips and assay protocol were the same as described in Example VIII. Each colloid containing anti-E1G antibody from Example X and Example XI was diluted to provide an optical density of 4 (wavelength at 560 nm) in MES/casein diluent (0.1 M MES, pH 6.1, 5% hydrolyzed casein, 0.024% Triton® surfactant, 0.04% bovine IgG, 0.1% Proclin-300® preservative) for wet mode testing. FIG. 7 shows two dose response curves for two kinds of colloids containing anti-E1G antibody linked via polymeric spacer arms at ambient temperature (22 °C). FIG. 8 shows three dose-response curves generated at three temperatures, 13 °C, 22 °C, and 37 °C, with the colloid of Example X. FIG. 8 indicates that the dose-response of anti-E1G antibody immobilized on polypyrrole colloid via the polymeric spacer arm varied little with respect to temperature when used in the wet mode type of assay.

Various modifications and alterations of this invention will become apparent to those skilled in the art without departing from the scope and spirit of this invention, and it should be understood that this invention is not to be unduly limited to the illustrative embodiments set forth herein.

What is claimed is:

1. A label comprising a colloidal particle having a spacer arm attached thereto and a specific binding member attached to the spacer arm.

2. The label of claim 1, wherein said colloidal particle is formed from a polymeric material.

3. The label of claim 2, wherein said colloidal particle is formed from polypyrrole.

4. The label of claim 1, wherein said colloidal particle is formed from a member selected from the group consisting of an aggregate dye particle, a metallic sol, and a non-metallic sol.

5. The label of claim 1, wherein said spacer arm is a biological linking group.

6. The label of claim 1, wherein said spacer arm is a synthetic polymeric linking group.

7. The label of claim 6, wherein said synthetic polymeric linking group is selected from the group consisting of polymers having vinyl alcohol units, polymers having alkylene glycol units, polymers having peptide units, polymers having saccharide units, polymers having nucleotide units, polymers having acrylate units, and polymers having amide units.

8. The label of claim 1, wherein said specific binding member is an antibody.

9. The label of claim 8, wherein said specific binding member is anti-E1G antibody.

10. The label of claim 1, wherein said specific binding member is an antigen.

11. The label of claim 1, wherein said specific binding member is a hapten.

12. The label of claim 1, wherein said label provides a dose response curve that varies only slightly over a wide temperature range when used in immunochromatographic assay strips.

13. The label of claim 11, wherein said wide temperature range is from about 13 °C to about 37 °C.

14. A method for determining the concentration of an analyte in a test sample, said method comprising the steps of:

a. contacting said test sample with the label of claim 1;

b. allowing said label to flow through a solid phase material that has a capture reagent immobilized in a capture zone;

c. detecting the amount of said label captured at said capture zone; and

d. determining the concentration of analyte in said test sample by correlating the amount of said label detected at said capture zone with the concentration of analyte in said test sample.

15. The method of claim 14, wherein said solid phase material is a porous strip material.

16. The method of claim 15, wherein said porous strip material is selected from the group consisting of nitrocellulose, paper, and glass fiber.

17. The method of claim 14, wherein said capture reagent is a conjugate comprising an antibody and a hapten.

18. The method of claim 14, wherein said capture reagent is a conjugate comprising an antigen and an antibody.

19. The method of claim 18, wherein said antigen is E1G.

20. Assay strip comprising:

(1) a strip of porous material having two ends;

(2) a sample mixing zone overlapping one end of said strip of porous material and containing the label of claim 1;

(3) a first capture zone containing a first capture reagent disposed on said strip of porous material and located at a distance from said sample mixing zone, said first capture reagent containing a binding member capable of specifically binding with said specific binding member of said label.

21. The assay strip of claim 20, further including a second capture zone containing a second capture reagent disposed on said strip of porous material and located at a distance from said sample mixing zone, said second capture reagent containing a binding member capable of specifically binding with said specific binding member of said label.

22. The assay strip of claim 20, further including a transparent laminate covering said strip of porous material and partially covering said sample mixing zone.

23. The assay strip of claim 20, further including a sheet of material for holding said strip of porous material and said sample mixing zone in place.

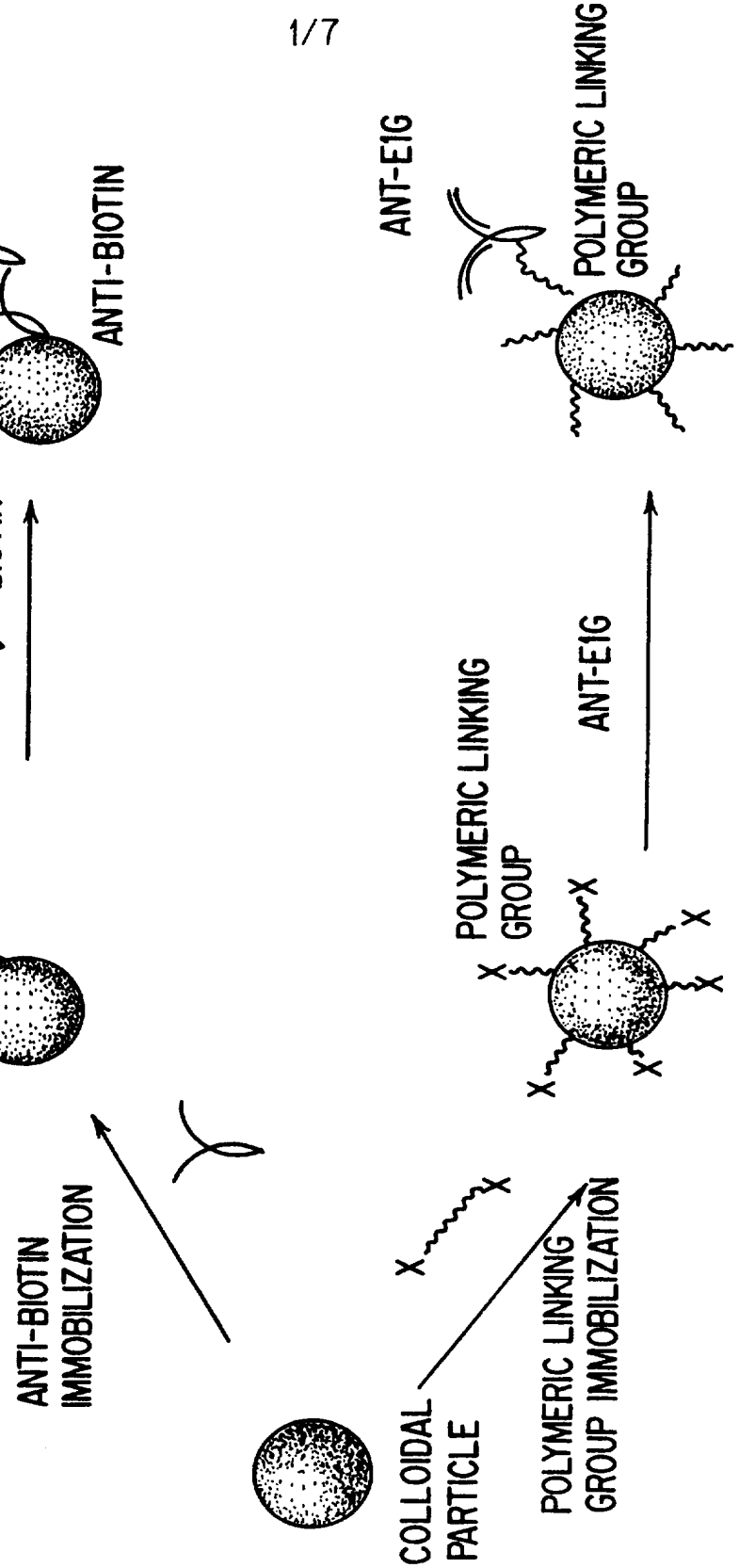

ANTI-E1G
ANTI-BIOTIN
ANTI-E1G
BIOTIN
ANTI-BIOTIN
ANT-E1G
POLYMERIC LINKING GROUP
POLYMERIC LINKING GROUP
ANT-E1G
X
ANTI-BIOTIN IMMOBILIZATION
COLLOIDAL PARTICLE
POLYMERIC LINKING GROUP IMMOBILIZATION

FIG.1

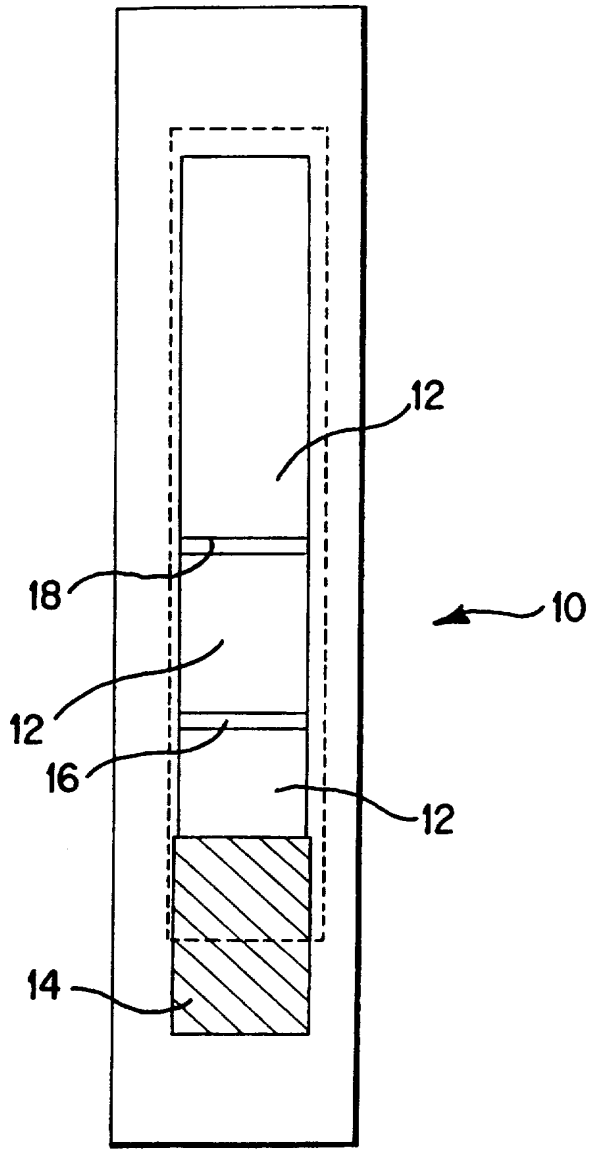


FIG. 2

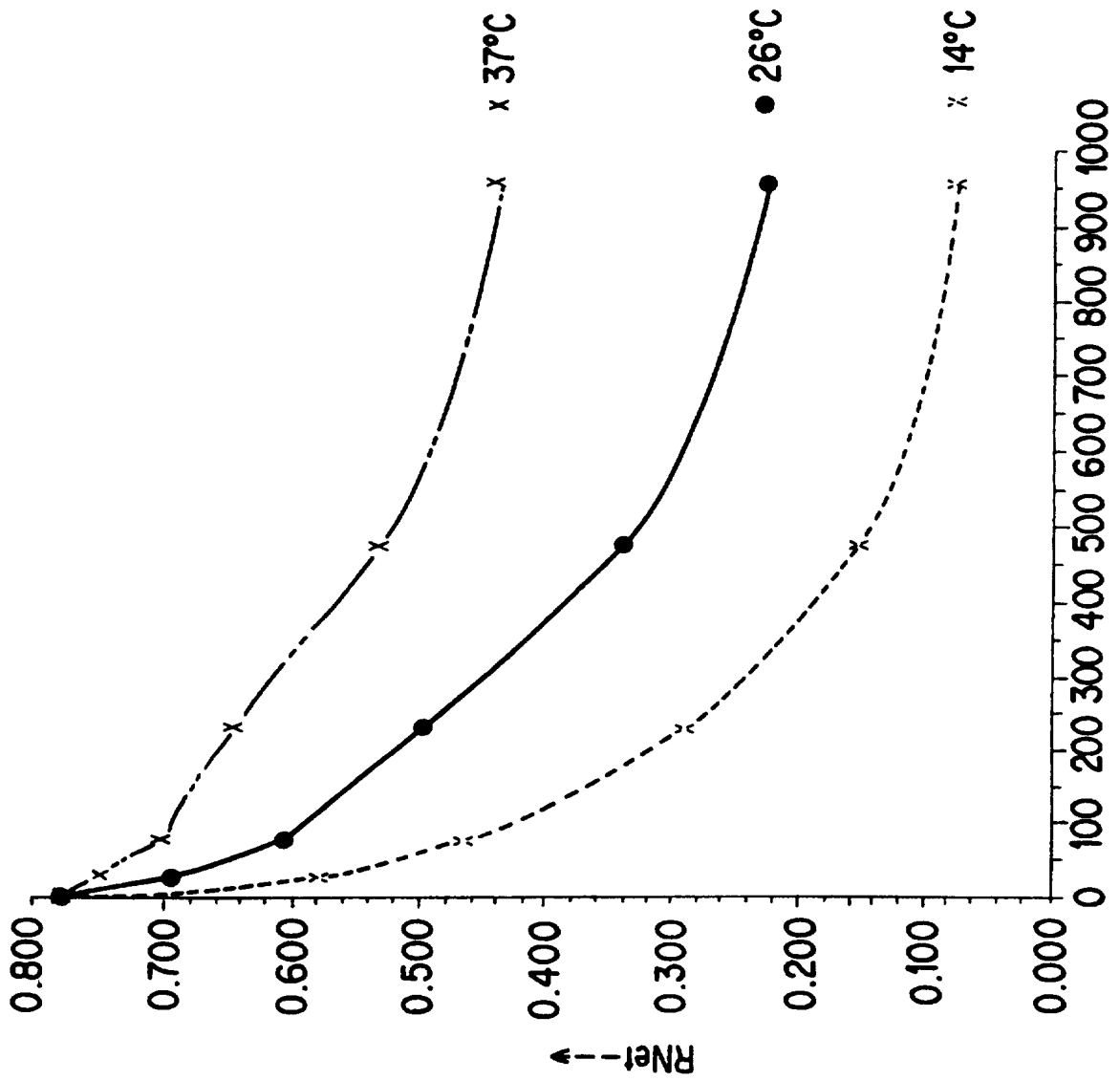

x 37°C
● 26°C
× 14°C
RNet→
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
0 100 200 300 400 500 600 700 800 900 1000
EIG nM→

FIG.3

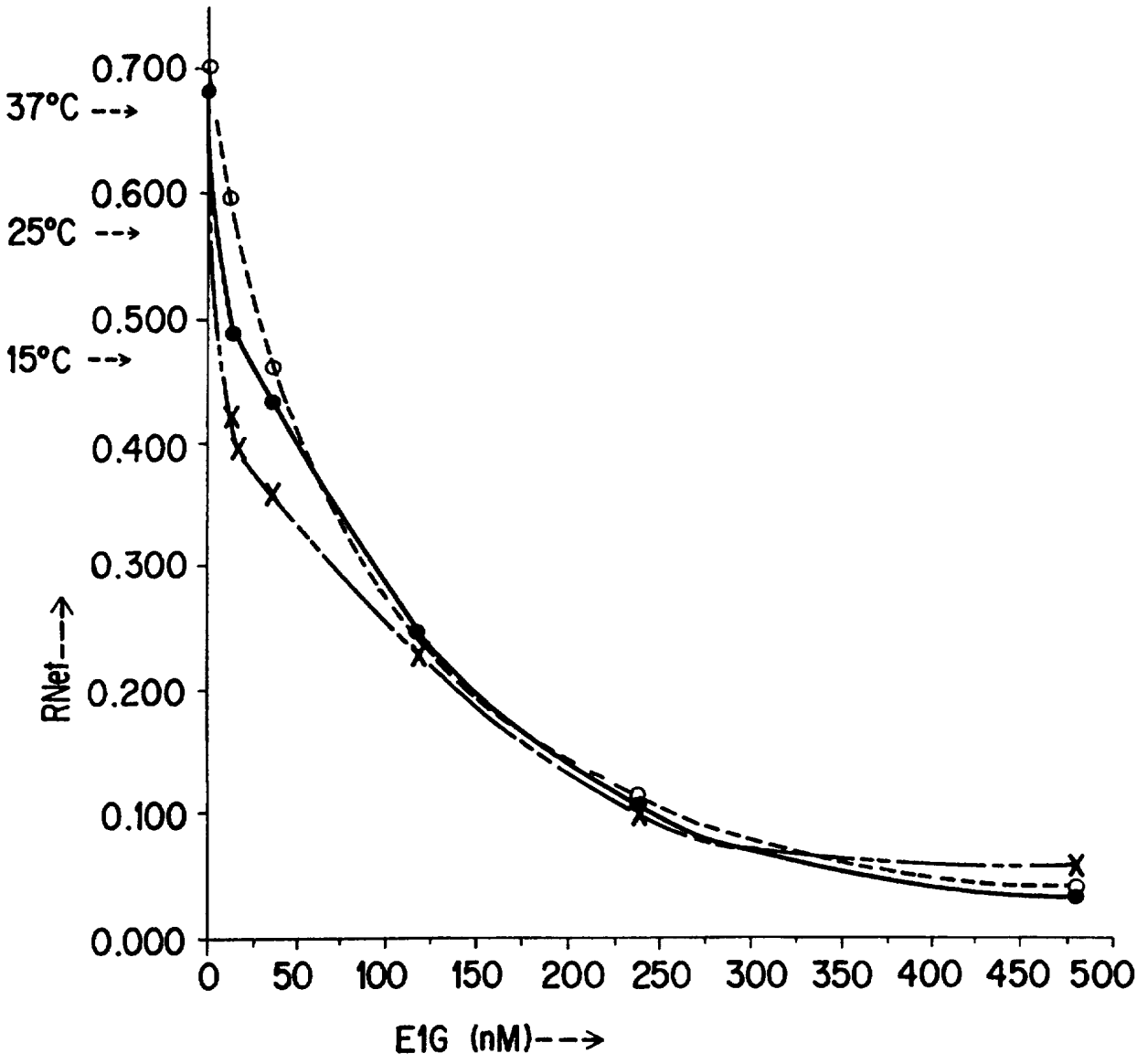

0.700
37°C -->
0.600
25°C -->
0.500
15°C -->
0.400
0.300
RNet-->
0.200
0.100
0.000
0
50
100
150
200
250
300
350
400
450
500
E1G (nM)-->

FIG. 4

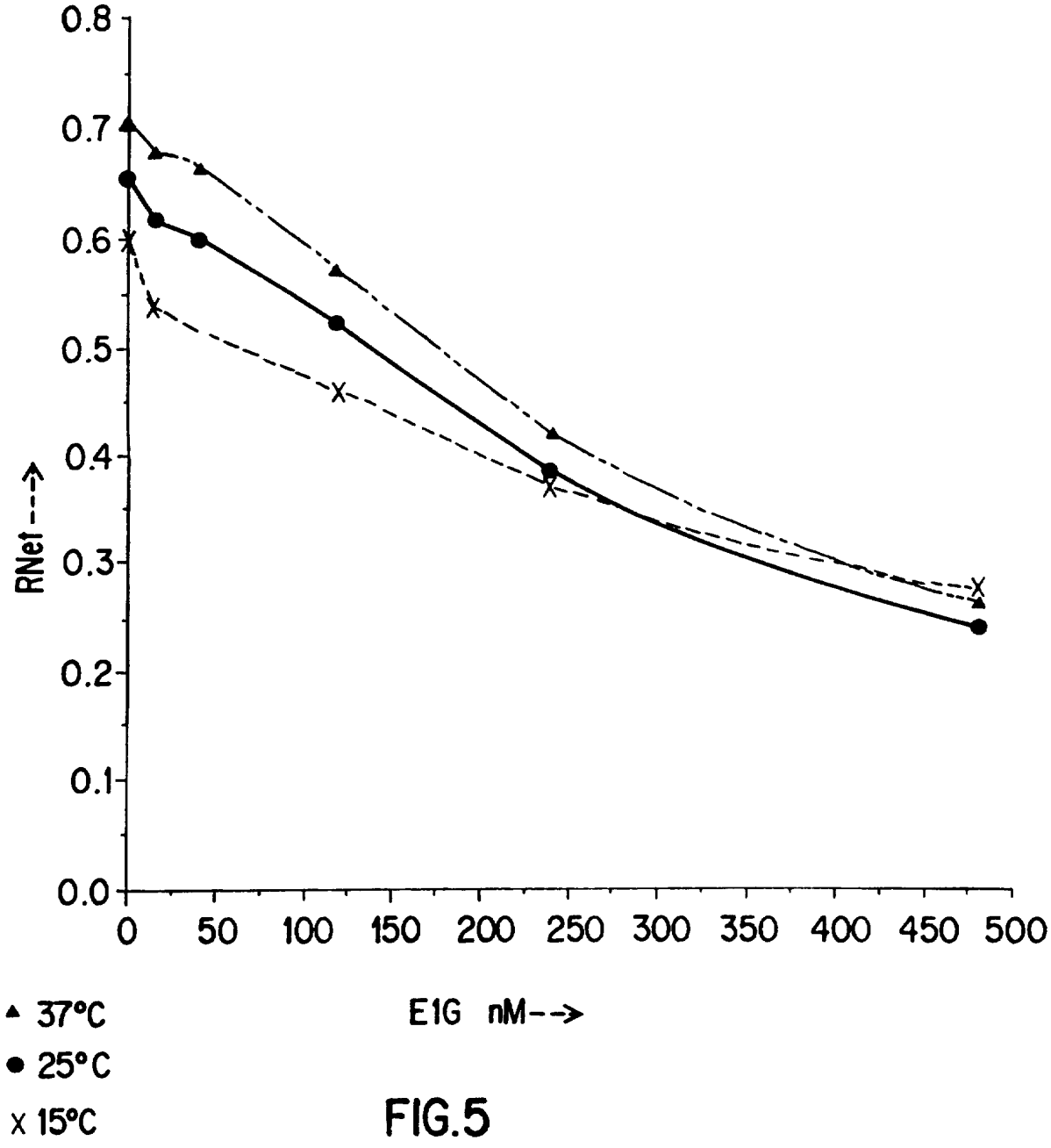
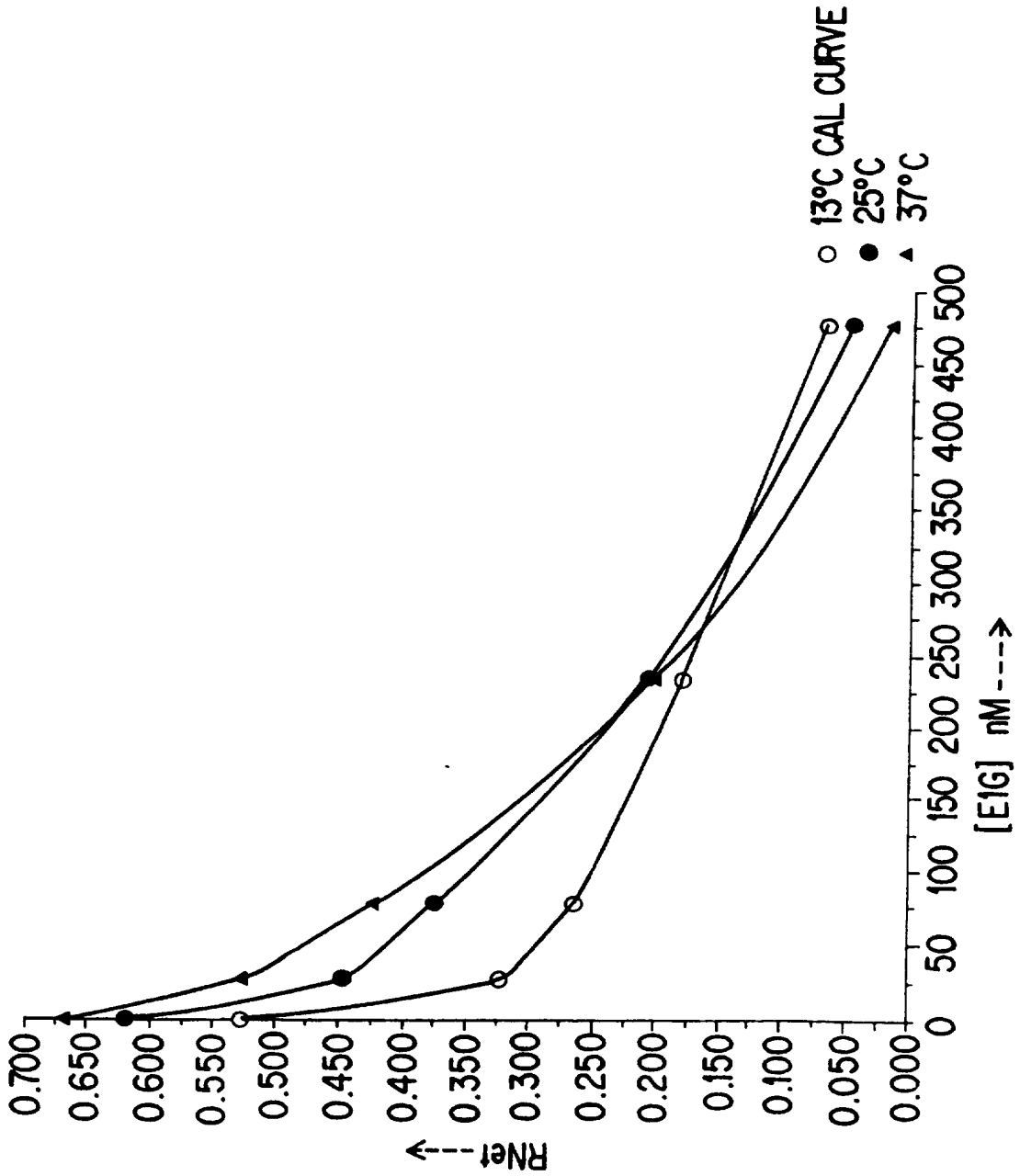

0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
RNet-->
0
50
100
150
200
250
300
350
400
450
500
E1G nM-->
▲ 37°C
● 25°C
X 15°C

FIG.5


13°C CAL CURVE
25°C
37°C
RNet --->
0.700
0.650
0.600
0.550
0.500
0.450
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
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0
50
100
150
200
250
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400
450
500
[E1G] nM -->

FIG.6

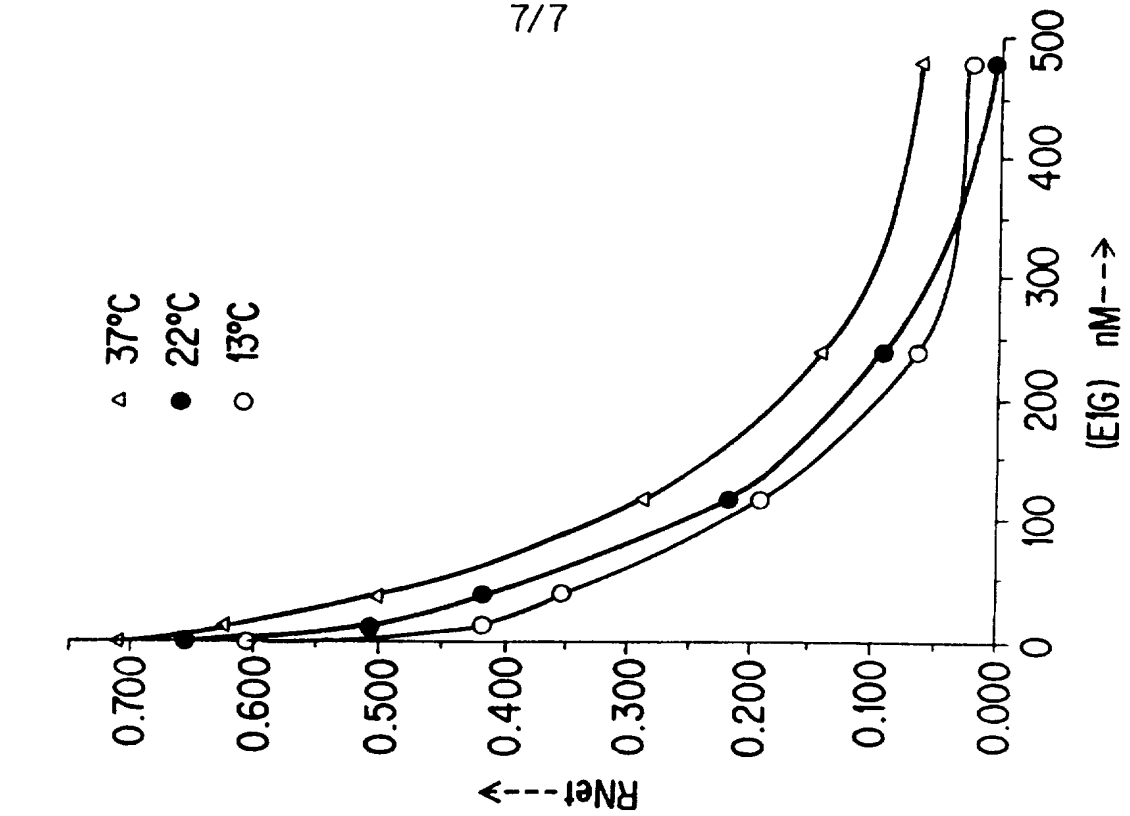
37°C
22°C
13°C
RNet
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
0
100
200
300
400
500
(E1G) nM

FIG.8

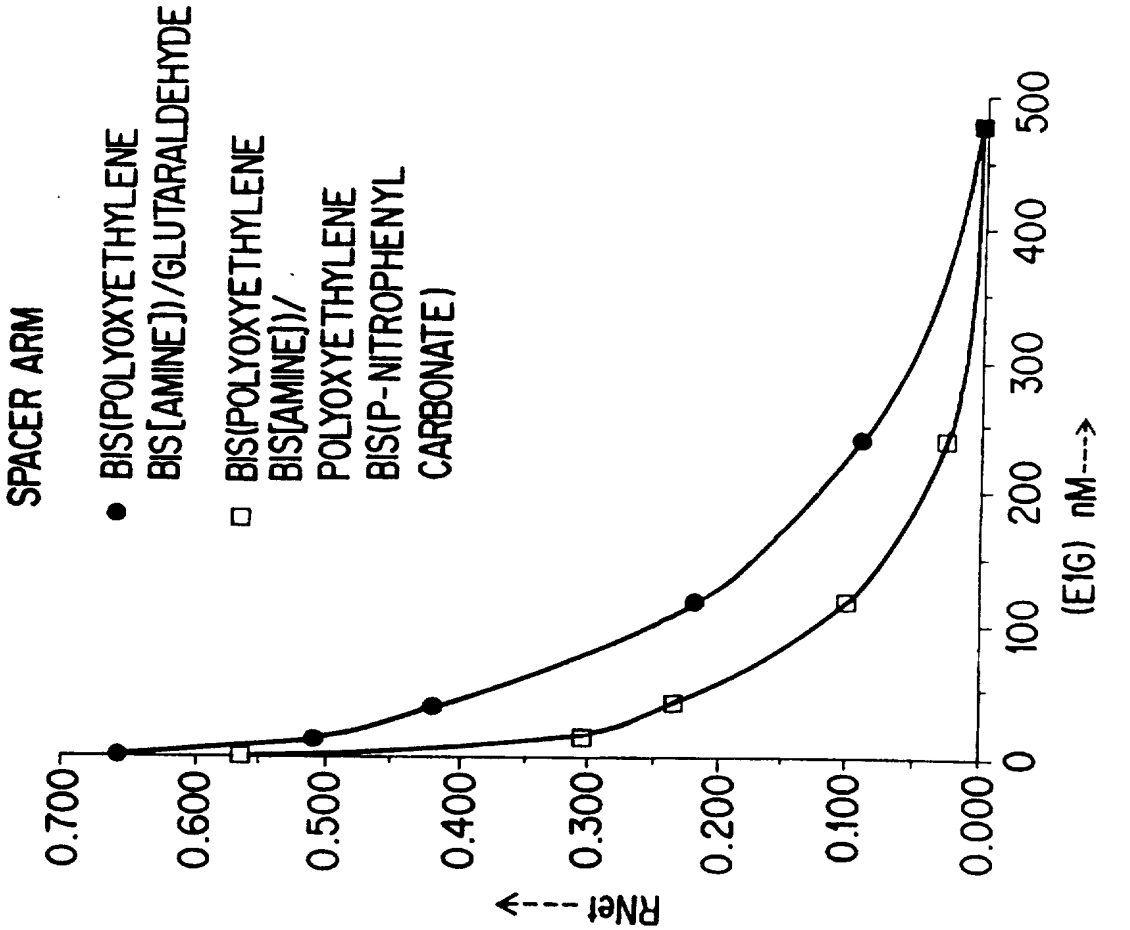
SPACER ARM
BIS(POLYOXYETHYLENE BIS[AMINE])/GLUTARALDEHYDE
BIS(POLYOXYETHYLENE BIS[AMINE])/ POLYOXYETHYLENE BIS(P-NITROPHENYL CARBONATE)
RNet
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
0
100
200
300
400
500
(E1G) nM

FIG.7

INTERNATIONAL SEARCH REPORT

Interna .l Application No
PCT/US 97/03886

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/58 G01N33/547

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 428 412 A (ORTHO DIAGNOSTIC SYSTEMS INC) 22 May 1991 see the whole document ---	1,4,5,8, 10,11, 14-18
X	EP 0 321 008 A (HOLLAND BIOTECHNOLOGY ;STICHTING PLANTENVEREDEL (NL)) 21 June 1989 see the whole document ---	1,4-8
X	WO 90 11128 A (VINTEN JOERGEN C AA) 4 October 1990 see the whole document --- -/--	1,4,5,8, 10,14-18

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 June 1997	08 -07- 1997

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Ceder, O

Form PCT/ISA/210 (second sheet) (July 1992)

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		AT 133788 T	15-02-96
		AU 1893388 A	27-04-89
		CA 1306675 A	25-08-92
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		DE 3854951 D	14-03-96
		DE 3854951 T	07-11-96
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		JP 7060159 B	28-06-95
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EP 0327843 A	16-08-89	US 4952517 A	28-08-90
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